Supplemental Methods

TFF2 inhibits tumour development and is a target for epigenetic silencing in gastric cancer

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Quantitative gene expression analysis

For Quantitative (Q) RT-PCR, oligo-dT primed cDNA was synthesised from 3μg total RNA using Murine Moloney Leukaemia Virus (MMLV) reverse transcriptase (Promega). QRT-PCR was performed on an ABI Prism® 7500 Real Time PCR System using SYBR green master mix (Applied Biosystems) according to the manufacturer's protocols. Relative gene expression values were obtained by normalization to the reference gene *Rpl32* (mouse) or *GAPDH* (human) using the -$2^{\Delta\Delta Ct}$ method, where -$2^{\Delta\Delta Ct}$ = ΔCt sample - ΔCt calibrator (Applied Biosystems). Primer sequences for QRT-PCR were designed using the primer3 package (***http://frodo.wi.mit.edu/primer3/***) and are listed in the tables below.

QRT-PCR primer sequences (mouse)

Gene	Forward primer sequence	Reverse primer sequence
Tff1	5'-AGAGGTTGCTGTTTTGATG-3'	5'-AGTCTGAGGGGTTGAACTG-3'
Tff2	5'-CCCCACAACAGAAAGAAC-3'	5'-GGGCACTTCAAAGATCAG-3'
Gkn1	5'-CTTCAGGACCTCGATACAATGG-3'	5'-TTGAGTACAAAGGCTGGTTTGG-3'
Gkn2	5'-AATGTAGACGGAAGTGGACAGC-3'	5'-GCATCCTTGTTCATTCTGTGC-3'
Gastrin	5'-AATGTAGACGGAAGTGGACAGC-3'	5'-GCATCCTTGTTCATTCTGTGC-3'
Somatostatin	5'-CCCAGACTCCGTCAGTTTCTG-3'	5'-GGGCATCATTCTCTGTCTGGTT-3'
H^+K^+-atpase	5'-CCGGTGGGTGTGGATCAG-3'	5'-GCAAAGAGCCCGGTCATG-3'
IL1-a	5'-AACCCATGATCTGGAAGAGACC-3'	5'-TGGTGCTGAGATAGTGTTTGTCC-3'
IL1-b	5'-CAGGCAGTATCACTCATTGTGG-3'	5'-GTGCAGTTGTCTAATGGGAACG-3'
IFN-g	5'TCAAGTGGCATAGATGTGGAAGAA-3'	5'-TGGCTCTGCAGGATTTTCATG-3'
Rpl32	5'GAGGTGCTGCTGATGTGC-3'	5'GGCGTTGGGATTGGTGACT-3'

QRT-PCR primer sequences (human)

Gene	Forward primer sequence	Reverse primer sequence
TFF1	5'-CCCTGGTGCTTCTATCCTAATAC-3'	5'-GAGCAGTCAATCTGTGTTGTGAGC-3'
TFF2	5'-TCTCAGACCGAAGAAACTGTGG-3'	5'-GGAACCAGCCTCTCTTAGTAATGG-3'
GAPDH	5'-GACATCAAGAAGGTGGTGAAGC-3'	5'-GTCCAACCCTGTTGCTGTAG-3'

Bisulphite PCR amplification for EPITYPER methylation analysis

To generate promoter fragments for EPITYPER methylation analysis, bisulphite converted DNA samples were amplified by nested PCR using HotStarTaq DNA polymerase (Qiagen). Methylation primer sequences were designed using MethPrimer (*http://www.urogene.org/methprimer/index1.html*) or Sequenom EpiDesigner (*http://www.epidesigner.com*) and are listed below along with relevant cycling parameters below. First round amplification used 1μL bisulphite DNA sample as template. Second round amplification used 1μL of the first round reaction product as template. Where present, 10-mer tags (bold type) and T7 promoter sequences (underlined) are indicated in the relevant primer sequences.

Human *TFF2* promoter:

First round primers	**Sequence**
TFF2-METH-OUT-F	5'-TTTTGTTTATTTTATTAATAGAAATGTATAGT-3'
TFF2-METH-OUT-R	5'-CCAAAACACATAACCCCAAAAC-3'
Second round primers	**Sequence**
10mer-hTFF2-METH-IN-F	5'-**AGGAAGAGAG**TTGTTTAGGGTAGGAAGAGGTATTAT-3'
T7-hTFF2-METH-IN-R	5'-CAGTAATACGACTCACTATAGGGAGAAGGCTCAAAAC ACATAACCCCAAAAC-3'

Cycling parameters:

First round:
95°C for 15 mins
25 cycles:
95°C for 30 secs
56°C for 1 min
72°C for 1 min

Second round:
95°C for 15 mins
25 cycles:
95°C for 30 secs
56°C for 1 min
72°C for 1 min
(final extension) 72˚C for 5 mins

Correct amplification was verified by performing electrophoresis of 5μL aliquots of each second round reaction through 1% Agarose, Tris-acetate-EDTA (TAE) gels. Products were visualised with the SYBR-safe DNA stain (Applied Biosystems).

Mouse *Tff2* promoter:

First round primers	**Sequence**
mTff2-METH-OUT-F2	5'- TGTGATTTTGTGGGTGTTTTATTTTA-3'
mTff2-METH-OUT-R2	5'- AACTAACTCAAATCCTACCTTTCACC-3'
Second round primers	**Sequence**
10mer-mTff2-METH-IN-F2	5'-**AGGAAGAGAG**TTTTGTGGGTGTTTTATTTTAGTTGT-3'
T7-mTff2-METH-IN-R2	5'-CAGTAATACGACTCACTATAGGGAGAAGGCTCCTA CCTTTCACCTACTAAATCTCCA-3'

Cycling parameters:

First round: 95°C for 15 mins
22 cycles:
95°C for 30 secs
56°C for 1 min
72°C for 1 min

Second round: 95°C for 15 mins
22 cycles:
95°C for 30 secs
56°C for 1 min
72°C for 1 min,
(final extension) 72˚C for 5 mins

Correct amplification was verified as described for the human *TFF2* assay.

Antibodies, immunohistochemistry and immunoblotting

Immunohistochemistry with peroxidase detection was done as described.[1] Sources of antibodies and lectins: rabbit polyclonal anti-recombinant human TFF2 (6-4, custom) diluted 1:2000[2]; rabbit polyclonal anti-mouse intrinsic factor (IF) (gift from Dr. David Alpers) diluted 1:500; mouse monoclonal anti-human Ki67 (DAKO) diluted 1:100; activated Caspase 3 (Cell Signaling Technology, MA; # 9661) diluted 1:200; F4/80 (Abd Serotec) diluted (1:300); myeloperoxidase (MPO) (Sapphire biosciences); rabbit polyclonal anti-mouse Tff1 (custom) diluted 1:1000[3]; lectin *GS*-II from *Griffinia simplicifolia* (EY Labs) used at 10µg/mL. For immunoblotting, tissue protein extracts were size fractionated by 15% SDS-PAGE, transferred to nitrocellulose membranes (GE Healthcare) and blocked in 5% non-fat milk powder/Tris-buffered saline (TBS) pH 7.4, 0.1% Tween-20. Membranes were incubated with primary antibodies overnight at 4°C: rabbit polyclonal anti-human STAT3 (#9132) diluted 1:1000; rabbit polyclonal anti-human phosphorylated STAT3 at tyrosine 705 (#9135) diluted 1:500; rabbit polyclonal anti-human ERK1/2 (#9102) diluted 1:1000; rabbit polyclonal anti-human phosphorylated ERK1/2 at threonine 202, tyrosine 204 (#9101) diluted 1:1000 (all Cell Signaling Technology); rabbit polyclonal anti-recombinant human TFF2 (6-4, custom) diluted 1:5000; rabbit polyclonal anti-GAPDH (Abcam, #ab9485) 1:3000. Detection was performed with swine polyclonal anti-rabbit IgG-HRP conjugates (DAKO) and enhanced chemiluminescence reagents (GE Healthcare).

Supplemental Methods References

1. Menheniott, TR, Woodfine K, Schulz R, et al. Genomic imprinting of *Dopa decarboxylase* in heart and reciprocal allelic expression with neighboring *Grb10*. Mol Cell Biol 2008;28: 386-96.

2. Srivatsa, G, Giraud AS, Ulaganathan M, et al. Biliary epithelial trefoil peptide expression is increased in biliary diseases. Histopathology 2002;40:261-8.

3. Cook, GA, Yeomans ND, Giraud AS. Temporal expression of trefoil peptides in the TGF-alpha knockout mouse after gastric ulceration. Am J Physiol 1997; 272: G1540-9.